

## WE CLAIM:

1. A conjugate comprising a bacterial superantigen and an antibody moiety, wherein

the superantigen is a low titer superantigen comprising regions A to E, which region A is a TCR binding site, and regions B to E determine the binding to MHC class II molecules; and

the amino acid sequence of the superantigen is substituted so that no more than 15 amino acid residues in region A are replaced with different amino acids, such that the substituted superantigen has reduced seroreactivity compared to the superantigen from which it is derived;

and wherein the antibody moiety is a full length antibody or any other molecule binding antibody active fragment, which is directed against a cancer-associated cell surface structure.

2. The conjugate of claim 1, wherein the superantigen is selected from the group consisting of staphylococcal enterotoxin (SE), a *Streptococcus pyogenes* exotoxin (SPE), a *Staphylococcus aureus* toxic shock-syndrome toxin (TSST-1), a streptococcal mitogenic exotoxin (SME) and a streptococcal superantigen (SSA).
3. The conjugate of claim 2, wherein the staphylococcal enterotoxin is staphylococcal enterotoxin A (SEA).
4. The conjugate of claim 2, wherein the staphylococcal enterotoxin is staphylococcal enterotoxin E (SEE).
5. The conjugate of claim 4, wherein the amino acid residue positions in region A to be replaced are selected from the group consisting of 20, 21, 24, 27, 173 and 204.
6. The conjugate of claim 5 further comprising substitutions of no more than 15 amino acid residues in region C.
7. The conjugate of claim 6, wherein the mutations in region C occur at the amino acid residue positions selected from the group consisting of 79, 81, 83 and 84.

8. The conjugate of claim 7 further comprising substitutions of no more than 15 amino acid residues in region E.
9. The conjugate of claim 8, wherein the mutation is at amino acid residue position 227.
10. A conjugate comprising a bacterial superantigen and an antibody moiety, wherein

the superantigen is a low titer superantigen comprising regions A to E, which region A is a TCR binding site, and regions B to E determine the binding to MHC class II molecules; and

the amino acid sequence of the superantigen is substituted so that no more than 15 amino acid residues in region B are replaced with different amino acids, such that the substituted superantigen has reduced seroreactivity compared to the superantigen from which it is derived;

and wherein the antibody moiety is a full length antibody or any other molecule binding antibody active fragment, which is directed against a cancer-associated cell surface structure.

11. The conjugate of claim 10, wherein the superantigen is selected from the group consisting of staphylococcal enterotoxin (SE), a *Streptococcus pyogenes* exotoxin (SPE), a *Staphylococcus aureus* toxic shock-syndrome toxin (TSST-1), a streptococcal mitogenic exotoxin (SME) and a streptococcal superantigen (SSA).
12. The conjugate of claim 11, wherein the staphylococcal enterotoxin is staphylococcal enterotoxin A (SEA).
13. The conjugate of claim 11, wherein the staphylococcal enterotoxin is staphylococcal enterotoxin E (SEE).
14. The conjugate of claim 13, wherein the amino acid residue positions in region B to be replaced are selected from the group consisting of 34, 35, 39, 40, 41, 42, 44, 45 and 49.

15. A conjugate comprising a bacterial superantigen and an antibody moiety, wherein

the superantigen is a low titer superantigen comprising regions A to E, which region A is a TCR binding site, and regions B to E determine the binding to MHC class II molecules; and

the amino acid sequence of the superantigen is substituted so that no more than 15 amino acid residues in region C are replaced with different amino acids, such that the substituted superantigen has reduced seroreactivity compared to the superantigen from which it is derived;

and wherein the antibody moiety is a full length antibody or any other molecule binding antibody active fragment, which is directed against a cancer-associated cell surface structure.

16. The conjugate of claim 15, wherein the superantigen is selected from the group consisting of staphylococcal enterotoxin (SE), a *Streptococcus pyogenes* exotoxin (SPE), a *Staphylococcus aureus* toxic shock-syndrome toxin (TSST-1), a streptococcal mitogenic exotoxin (SME) and a streptococcal superantigen (SSA).
17. The conjugate of claim 16, wherein the staphylococcal enterotoxin is staphylococcal enterotoxin A (SEA).
18. The conjugate of claim 16, wherein the staphylococcal enterotoxin is staphylococcal enterotoxin E (SEE).
19. The conjugate of claim 18, wherein the amino acid residue positions in region C to be replaced are selected from the group consisting of 74, 75, 78, 79, 81, 83 and 84,
20. The conjugate of claim 19 further comprising substitutions of no more than 15 amino acid residues in region A.
21. The conjugate of claim 20, wherein the substitutions in region A occur at the amino acid residue positions selected from the group consisting of 20, 21, 24, 27, 173 and 204.
22. The conjugate of claim 21 further comprising substitutions of no more than 15 amino acid residues in region E.

23. The conjugate of claim 22, wherein the mutation is at amino acid residue position 227.
24. The conjugate of claim 23, wherein the SEE amino acid sequence includes the substitutions of R20G, N21T, S24G, R27K, K79E, K81E, K83S, K84S and D227S.
25. The conjugate of claim 23, wherein the SEE amino acid sequence includes the substitutions of R20G, N21T, S24G, R27K, K79E, K81E, K83S, K84S and D227A.
26. The conjugate of claim 22, wherein the superantigen has the amino acid sequence of SEQ ID NO: 2.
27. The conjugate of claim 15, wherein the antibody moiety is a Fab fragment.
28. The conjugate of claim 27, wherein the Fab fragment is C215Fab.
29. The conjugate of claim 27, wherein the Fab fragment is 5T4Fab.
30. The conjugate of claim 29, wherein the superantigen has the amino acid sequence of SEQ ID NO: 1.
31. The conjugate of claim 27 further comprising a cytokine.
32. The conjugate of claim 30, wherein the cytokine is an interleukin.
33. The conjugate of claim 31, wherein the interleukin is IL2 or a derivative thereof having essentially the same biological activity of native IL2.
34. The conjugate of claim 15, wherein said cancer is selected from the group consisting of lung, breast, colon, kidney, pancreatic, ovarian, stomach, cervix and prostate cancer.
35. A conjugate comprising a bacterial superantigen and an antibody moiety, wherein  
the superantigen is a low titer superantigen comprising regions A to E, which region A is a TCR binding site and regions B to E determine the binding to MHC class II molecules; and

the amino acid sequence of the superantigen is substituted so that no more than 15 amino acid residues in region D are replaced with different amino acids, such that the substituted superantigen has reduced seroreactivity compared to the superantigen from which it is derived;

and wherein the antibody moiety is a full length antibody or any other molecule binding antibody active fragment, which is directed against a cancer-associated cell surface structure.

36. The conjugate of claim 34, wherein the superantigen is selected from the group consisting of staphylococcal enterotoxin (SE), a *Streptococcus pyogenes* exotoxin (SPE), a *Staphylococcus aureus* toxic shock-syndrome toxin (TSST-1), a streptococcal mitogenic exotoxin (SME) and a streptococcal superantigen (SSA).
37. The conjugate of claim 36, wherein the staphylococcal enterotoxin is staphylococcal enterotoxin A (SEA).
38. The conjugate of claim 36, wherein the staphylococcal enterotoxin is staphylococcal enterotoxin E (SEE).
39. The conjugate of claim 37, wherein the amino acid residue positions in region D to be replaced are selected from the group consisting of 187, 188, 189 and 190.
40. A conjugate comprising a bacterial superantigen and an antibody moiety, wherein

the superantigen is a low titer superantigen comprising regions A to E, which region A is a TCR binding site, and regions B to E determine the binding to MHC class II molecules; and

the amino acid sequence of the superantigen is substituted so that no more than 15 amino acid residues in region E are replaced with different amino acids, such that the substituted superantigen has reduced seroreactivity compared to the superantigen from which it is derived;

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and wherein the antibody moiety is a full length antibody or any other molecule binding antibody active fragment, which is directed against a cancer-associated cell surface structure.

41. The conjugate of claim 39, wherein the superantigen is selected from the group consisting of staphylococcal enterotoxin (SE), a *Streptococcus pyogenes* exotoxin (SPE), a *Staphylococcus aureus* toxic shock-syndrome toxin (TSST-1), a streptococcal mitogenic exotoxin (SME) and a streptococcal superantigen (SSA).
42. The conjugate of claim 41, wherein the staphylococcal enterotoxin is staphylococcal enterotoxin A (SEA).
43. The conjugate of claim 41, wherein the staphylococcal enterotoxin is staphylococcal enterotoxin E (SEE).
44. The conjugate of claim 42, wherein the amino acid residue positions in region E to be replaced are selected from the group consisting of 217, 220, 222, 223, 225 and 227.
45. The conjugate of claim 43 further comprising substitutions of no more than 15 amino acid residues in region A.
46. The conjugate of claim 44, wherein the substitutions in region A occur at the amino acid residue positions selected from the group consisting of 20, 21, 24, 27, 173 and 204.
47. The conjugate of claim 45 further comprising substitutions of no more than 15 amino acid residues in region B.
48. The conjugate of claim 46, wherein the substitutions in region B occurs at the amino acid residue positions selected from the group consisting of 34, 35, 39, 40, 41, 42, 44, 45 and 49.
49. The conjugate of claim 47 further comprising substitutions of no more than 15 amino acid residues in region C.

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50. The conjugate of claim 48, wherein the substitutions in region C occurs at the amino acid residue positions selected from the group consisting of 74, 75, 78, 79, 81, 83 and 84.
51. The conjugate of claim 49 further comprising substitutions of no more than 15 amino acid residues in region D.
52. The conjugate of claim 50, wherein the substitutions in region D occurs at the amino acid residue positions selected from the group consisting of 187, 188, 189 and 190.
53. A pharmaceutical composition comprising a therapeutically effective amount of a conjugate, wherein said conjugate comprises a bacterial superantigen and an antibody moiety, wherein
- the superantigen is a low titer superantigen comprising regions A to E, which region A is a TCR binding site, and regions B to E determine the binding to MHC class II molecules; and
- the amino acid sequence of the superantigen is substituted so that no more than 15 amino acid residues in region C are replaced with different amino acids, such that the substituted superantigen has reduced seroreactivity compared to the superantigen from which it is derived;
- and wherein the antibody moiety is a full length antibody or any other molecule binding antibody active fragment, which is directed against a cancer-associated cell surface structure.
54. The pharmaceutical composition of claim 52, wherein the superantigen is selected from the group consisting of staphylococcal enterotoxin (SE), a *Streptococcus pyogenes* exotoxin (SPE), a *Staphylococcus aureus* toxic shock-syndrome toxin (TSST-1), a streptococcal mitogenic exotoxin (SME) and a streptococcal superantigen (SSA).
55. The pharmaceutical composition of claim 54, wherein the staphylococcal enterotoxin is staphylococcal enterotoxin A (SEA).

56. The pharmaceutical composition of claim 54, wherein the staphylococcal enterotoxin is staphylococcal enterotoxin E (SEE).
57. The pharmaceutical composition of claim 55, wherein the amino acid residue positions in region C to be replaced are selected from the group consisting of 74, 75, 78, 79, 81, 83 and 84.
58. The pharmaceutical composition of claim 56 further comprising substitutions of no more than 15 amino acid residues in region A.
59. The pharmaceutical composition of claim 57, wherein the substitutions in region A occur at the amino acid residue positions selected from the group consisting of 20, 21, 24, 27, 173 and 204.
60. The pharmaceutical composition of claim 58 further comprising a substitutions of no more than 15 amino acid residues in region E.
61. The pharmaceutical composition of claim 59, wherein the mutation is at amino acid residue position 227.
62. The pharmaceutical composition of claim 60, wherein the SEE amino acid sequence includes the substitutions of R20G, N21T, S24G, R27K, K79E, K81E, K83S, K84S and D227S.
63. The pharmaceutical composition of claim 60, wherein the SEE amino acid sequence includes the substitutions of R20G, N21T, S24G, R27K, K79E, K81E, K83S, K84S and D227A.
64. The pharmaceutical composition of claim 59, wherein the superantigen has the amino acid sequence of SEQ ID NO: 2.
65. The pharmaceutical composition of claim 52, wherein the antibody moiety is a Fab fragment.
66. The pharmaceutical composition of claim 64, wherein the Fab fragment is C215Fab.
67. The pharmaceutical composition of claim 64, wherein the Fab fragment is 5T4Fab.



68. The pharmaceutical composition of claim 67, wherein the superantigen has the amino acid sequence of SEQ ID NO: 1.
69. The pharmaceutical composition of claim 64 further comprising a cytokine.
70. The pharmaceutical composition of claim 67, wherein the cytokine is an interleukin.
71. The pharmaceutical composition of claim 68, wherein the interleukin is IL2 or a derivative thereof having essentially the same biological activity of native IL2.
72. The pharmaceutical composition of claim 52, wherein said cancer is selected from the group consisting of lung, breast, colon, kidney, pancreatic, ovarian, stomach, cervix and prostate cancer.

73. A method of treating cancer in a mammal by activation of the immune system of said mammal comprising administering to said mammal a therapeutically effective amount of a conjugate, wherein said conjugate comprises a bacterial superantigen and an antibody moiety, wherein

the superantigen is a low titer superantigen comprising regions A to E, which region A is a TCR binding site, and regions B to E determine the binding to MHC class II molecules; and

the amino acid sequence of the superantigen is substituted so that no more than 15 amino acid residues in region C are replaced with different amino acids, such that the substituted superantigen has reduced seroreactivity compared to the superantigen from which it is derived;

and wherein the antibody moiety is a full length antibody or any other molecule binding antibody active fragment, which is directed against a cancer-associated cell surface structure.

74. The method of claim 71, wherein the superantigen is selected from the group consisting of staphylococcal enterotoxin (SE), a *Streptococcus pyogenes* exotoxin (SPE), a *Staphylococcus aureus* toxic shock-syndrome toxin (TSST-1), a streptococcal mitogenic exotoxin (SME) and a streptococcal superantigen (SSA).

75. The method of claim 74, wherein the staphylococcal enterotoxin is staphylococcal enterotoxin A (SEA).
76. The method of claim 74, wherein the staphylococcal enterotoxin is staphylococcal enterotoxin E (SEE).
77. The method of claim 74, wherein the amino acid residue positions in region C to be replaced are selected from the group consisting of 74, 75, 78, 79, 81, 83 and 84.
78. The method of claim 75 further comprising substitutions of no more than 15 amino acid residues in region A.
79. The method of claim 76, wherein the substitutions in region A occur at the amino acid residue positions selected from the group consisting of 20, 21, 24, 27, 173 and 204.
80. The method of claim 77 further comprising a substitutions of no more than 15 amino acid residues in region E.
81. The method of claim 78, wherein the mutation is at amino acid residue position 227.
82. The method of claim 79, wherein the SEE amino acid sequence includes the substitutions of R20G, N21T, S24G, R27K, K79E, K81E, K83S, K84S and D227S.
83. The method of claim 79, wherein the SEE amino acid sequence includes the substitutions of R20G, N21T, S24G, R27K, K79E, K81E, K83S, K84S and D227A.
84. The method of claim 78, wherein the superantigen has the amino acid sequence of SEQ ID NO: 2.
85. The method of claim 71, wherein the antibody moiety is a Fab fragment.
86. The method of claim 83, wherein the Fab fragment is C215Fab.
87. The method of claim 83, wherein the Fab fragment is 5T4Fab.
88. The method of claim 87, wherein the superantigen has the amino acid sequence of SEQ ID NO: 1.

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89. The method of claim 83 further comprising a cytokine.
90. The method of claim 86, wherein the cytokine is an interleukin.
91. The method of claim 87, wherein the interleukin is IL2 or a derivative thereof having essentially the same biological activity of native IL2.
92. The method of claim 71, wherein ~~said cancer~~ is selected from the group consisting of lung, breast, colon, kidney, pancreatic, ovarian, stomach, cervix and prostate cancer.

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